Journal of Applied Biology & Biotechnology Vol. 10(Suppl 2), pp. 91-99, June, 2022
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2022.10s210

Characterization of tannery effluents by analyzing the recalcitrant organic pollutants and phytotoxicity assay

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ARTICLE INFO

Article history:
Received on: September 30, 2021
Accepted on: March 09, 2022
Available online: June 20, 2022

Key words:
Tannery effluent,
Common effluent treatment plant,
Recalcitrant organic pollutants,
Gas chromatography–mass spectrometry,
Phytotoxicity

ABSTRACT

The tannery industries have greatly improved their treatment system; treated effluents still need to be properly delineated for contaminants and toxicity. In this study, the analysis of both raw and treated tannery effluents (TEs) revealed the maximum reduction of chromium (91%), followed by chemical oxygen demand (COD) (76.7%), total dissolved solids (TDSs) (43.3%), oil and grease (37.2%), and biological oxygen demand (BOD) (33.3%) after common effluent treatment plant (CETP) treatment. Further, the concentration of TDS (13,317 ± 2.7 mg/l), BOD (280 ± 4.47 mg/l), COD (409 ± 2.4 mg/l), sulfate (3773 ± 7.3 mg/l), nitrate (734.86 ± 0.4 mg/l), chloride (8053.59 ± 18.7 mg/l), and chromium (7.153 ± 0.02 mg/l) in treated TE was 6.3-, 9.3-, 1.6-, 3.8-, 73.4-, 13.4-, and 3.6-fold higher than the permissible limit fixed by Central Pollution Control Board. Gas chromatography–mass spectrometry analysis revealed the presence of recalcitrant organic pollutants such as furan, phthalate, and fatty acid in CETP-treated TE. Phytotoxicity investigation of TE on fenugreek (*Trigonella foenum-graecum* L.) and mung bean (*Vigna radiata* L.) seeds germination shows that both raw and CETP-treated TEs were inhibitory for seed germination and plant growth. Further, treated TE inhibited seed germination (30%), root length (97.3%), and shoot length (88.7%) in *T. foenum-graecum* and at 50% concentration, respectively. However, CETP-treated TE was less toxic than the raw TE. Further, fenugreek seeds were more sensitive to TE, as they could not be germinated in both undiluted raw and treated TEs. The finding of the present study reveals that CETP-treated effluents contain a complex mixture of toxic contaminants, indicating that it is not safe to discharge these effluents into the environment.

1. INTRODUCTION

Environmental pollution is one of the most serious global problems and it is increasing day by day due to industrial activities [1,2]. The tannery industries (TIs) play an important role in Indian exports and employment for people from the economically weaker population [3]. India is the second one maximum populous country in the world and about 15% of the Indian economy is especially primarily based on TIs [4]. India comes inside the 2nd rank with inside the pinnacle five leather-based manufacturing industries, generating almost 1.4 billion square feet of leather-based consistent with the year throughout the world (https://www.investindia.gov.in/sector/leather). TIs are also seen as one of the most polluting industries as they release huge volumes of hazardous wastewater along with toxic contaminants, and however, their sufficient treatment is the most difficult problem around the world [5,6]. The manufacturing process of leather in TIs uses huge amounts of water and various chemicals and produces the substances and express huge portions of toxic and extremely colored tannery effluent (TE) [7,8]. It uses more than 250 chemicals for leather production in TIs during tanning, dyeing, bleaching, washing, and finishing processes are often characterized by their dark brownish color, biological oxygen demand (BOD), chemical oxygen demand (COD), and total dissolved solid (TDS), etc. The previous researchers investigation was found that TE emitted by TIs had high levels of physicochemical parameters such as BOD (160–660 ± 45 mg/L), COD level (322–1428 ± 5.56 mg/L), TDS (3491–9700 ± 60 mg/L), and total chromium (5.71–6.88 mg/L) [5,9-11]. Mainly biological treatment processes like activated sludge process are applied by TIs to treat wastewater [11,12]. However, many toxic compounds such as chromium, dye, tannins, phenols, and organic acids cannot be removed completely by conventional methods due to their toxic effects and poor biodegradability which pose a threat to the aquatic environment [3]. Most of the TIs are made on a small scale and do not have sufficient treatment facilities. Thus, treated wastewater from TIs contains toxic metals such as chromium, lead, zinc, and nickel as well as toxic organic compounds and phenolic derivatives, which can cause toxic effects on living organisms [13]. Thus, wastewater treated with TIs contains inorganic pollutants such as chromium, lead, cadmium, zinc, and nickel, which are quite toxic to the environment. Subsequently, when contaminated wastewater comes

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into contact with aquatic resources, it inhibits the photosynthesis process and reduces the level of dissolved oxygen in aquatic systems, thereby affecting the flora and fauna as well as the aquatic ecosystem [14]. Chromium is very hazardous to the environment and has severe problems such as carcinogenic and mutagenic effects [15]. Because of this, various studies have confirmed the toxic and genotoxic effects of TE in the plant [7,16]. A toxicity test can be a useful procedure for organic and inorganic pollutants effects in industrial wastewater. Various biological indicators such as plants, animals and microorganisms have been frequently used to monitor pollutant toxicity on the environment. Plant growth bioassays have proven to be sensitive, cheap, and effective methods used to screening and assessment of environmental toxins [17]. The effect of various substances or pollutants on seed germination and subsequent plant growth is determined using a phytotoxicity test. The seeds and plants of fenugreek (*Trigonella foenum-graecum* L.) are known for multiple properties such as pharmacological uses antidiabetic, antibacterial, antifungal, and hypocholesterolemic and other uses in spices in Indian and Asian cuisine [18,19]. We have used seeds of fenugreek (*T. foenum-graecum* L.) for this study to see the effect of organic and inorganic pollutants in tannery wastewater on plants growth.

Therefore, the present study aimed to identify and characterize the toxic recalcitrant organic pollutants in raw (untreated) and treated TE performed at the common effluent treatment plant (CETP) in Unnao district, India. In addition, the toxicological effect of contaminants present in TE was investigated on plants test model using mung bean (*Vigna radiata* L.) and fenugreek (*T. foenum-graecum* L.), respectively.

2. MATERIALS AND METHODS

2.1. Collection of TE Samples

Sampling site, CETP, Unnao (UP), has a large number of tanning industries along the Ganges coast and is equipped with a craft tanning process and a two-stage beam house operation and tanning process. TE samples were collected in pre-sterilized polypropylene bottles from the before and after treatment drains tank of CETP. The collected effluent samples were appropriately labeled and brought to the laboratory in an icebox (4°C) and further kept at 4°C for analysis of physicochemical parameters, heavy metal analysis, organic compounds, as well phytotoxicity test.

2.2. Analysis of TE

2.2.1. Bacteriological analysis

Quantitative bacteriological analysis in collected CETP-treated TE samples was analyzed by serial dilution method by plating on plate count agar and MacConkey agar at 37 ± 2°C for enumeration of total heterotrophs and coliforms bacteria [20].

2.2.2. Antibiotic susceptibility assay

The antibiotic susceptibility testing of total heterotrophs was performed by disk diffusion method against the following antibiotics using gentamycin, ciprofloxacin, ampicillin, erythromycin, vancomycin, penicillin-G, nalidixic acid, amoxicillin, polymyxin-B, tetracycline, neomycin, amikacin, norfloxacin, chloramphenicol, and kanamycin using Mueller-Hinton agar medium (incubation at 30°C for 24 h) [17]. The inhibition zones were measured after 24 h and classified as resistant, intermediate, or susceptible according to DIFCO [21].

2.2.3. Physicochemical analysis

The TE samples were analyzed for various physicochemical parameters such as pH, COD, BOD, TDS, total suspended solids, total nitrogen, phosphate, nitrate, and sulfate according to standard methods of APHA [20]. These parameters were selected because they are considered harmful to the receiving environment and were included in the discharge limit. The heavy metals in samples were determined by the digestion method (nitric acid + perchloric acid; 100 ml [5:1]) on atomic absorption spectrometer (Analytik Jena, ZEEnit 700, Germany).

2.4. Detection of Recalcitrant Organic Pollutants from TE by Gas Chromatography–Mass Spectrometry (GC–MS)

The extraction of recalcitrant organic pollutants in both TE samples was done utilizing a liquid-liquid extraction procedure using mixed solvents systems for the GC–MS analysis [16,22]. TE samples (50 ml) were extracted with an equal volume (1:1) of dichloromethane (DCM) and ethyl acetate. The transparent layer of the solvent containing organic pollutants was separated and evaporated to dryness under vacuum at 45°C. The dried residual extract was mixed with DCM (2 ml) and filtered through a 0.45 µm pore size filter using 2 ml of the syringe. The samples were derivatized using TMS (BSTFA [N, O-bis (TMS) trifluoroacetamide] TMCS) [23]. An aliquot of 1 XL of silylated compounds was injected in PE Auto system XL gas chromatograph interfaced with a TurboMass mass spectrometric mass selective detector (Perkin Elmer, Waltham, MA, USA). The carrier gas was used as a helium gas with a flow rate of 1 ml/min. The column (50°C; for 5 min) was programmed to run 50–300°C at 10°C/min (hold time – 5 min). EI mass spectra in the range of 30–550 (m/z) at 70 eV were acquired in full-scan mode. The peak's identity was confirmed by comparing their mass spectra to that in the NIST (USA) library, included with the equipment.

2.5. Toxicological Analysis: Phytotoxicity on Mung Bean and Fenugreek

Mung bean (*V. radiata* L.) and fenugreek (*T. foenum-graecum* L.) seeds were used for the phytotoxicity studies. The seed germination test was conducted on filter paper (125 mm in diameter, Whatman No. 1) in Petri dishes (20 mm × 120 mm) followed by a layer of cotton on the bottom. Five test solutions (6.5, 12.5, 25, 50, and 100%, v/v) of the TE were prepared using tap water. Ten seeds were kept on filter paper in each Petri dish irrigated with the 10 ml of the test solutions, and then, the plates were incubated at 23 ± 1°C in the dark for 7 days. The experiment was conducted in triplicates. After 7 days, germinating seeds were counted and five randomly selected seedlings from each set were measured root and shoot length (cm) by centimeter scale. For biomass estimation, five seedlings from each Petri dish were dried in an oven at 70°C for 48 h and then weighed the dry weight which was expressed as biomass in gram (g). Plant growth index parameters such as germination %, relative seed germination %, germination index % (GI%), relative root growth % (RRG%), relative toxicity %, and % phytotoxicity were calculated using the following formula to determine the phytotoxicity of TE [24]. The phytotoxicity data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests.

$$Germination\ \% = \frac{No.\text{of seeds germinated}}{Total\ no.of\ seeds} \times 100 \quad (1)$$

$$Relative\ seed\ germination(RSG)\% = \frac{Treatment\ (no.of\ seeds\ germinated)}{Control\ (no.\ of\ seeds\ germinated)} \times 100 \quad (2)$$

$$Relative\,root\,growth\,(RRG)\% = \frac{Treatment\ (average\,root\,length)}{Control\ (average\,root\,length)} \times 100 \quad (3)$$

$$Germination\,index\,(GI)\% = \frac{RSG\% \times RRG\%}{100} \quad (4)$$

$$\%\,Phytotoxicity = \frac{Control\,(average\,root\,length) - Treatment\ (average\,root\,length)}{Control\ (average\,root\,length)} \times 100 \quad (5)$$

$$Relative\,toxicity\ \% = \frac{Control\,(average\,seedling\,length) - Treatment\ (average\,seedling\,length)}{Control\ (average\,seedling\,length)} \quad (6)$$

3. RESULTS AND DISCUSSION

3.1. Physicochemical Characteristics of TE

The wastewater discharge from tanneries is always characterized by a heavy pollution load. Also, small scale industries do not have their own waste water treatment plant which can effectively remove the load of pollutants before discharge. Therefore, CETP has been used for the treatment of hazardous tannery wastewater. To know the efficiency of CETP, we analyzed both raw and treated effluents for various physicochemical parameters including heavy metals. The measured physicochemical parameters of the effluent samples and Central Pollution Control Board (CPCB) [25] standard for the discharge limits of industrial wastewaters are given in Table 1.

The results showed reduction of BOD (33.3%), COD (76.68%), TDS (43.38%), and chromium (91.23%) after CETP treatment. The pH of the raw effluent was 7.24 ± 0.0 which was increased to pH (8.29 ± 0.1) after CETP treatment. The comparative analysis of analyzed parameters in both raw and treated effluents suggests insufficiency of CETP to complete treatment of TE. The effluent discharges by CETP found to contain high TDS (13317 ± 2.68 mg/l), BOD (280 ± 4.47 mg/l), COD (409 ± 2.37 mg/l), sulfate (3773.04 ± 7.29 mg/l), nitrate (734.86 ± 0.42 mg/l), and chloride (8053.59 ± 18.74 mg/l) and heavy metals such as chromium (7.2 ± 0.02 mg/l), Co (0.118 ± 0.01mg/l), Ni (0.265 ± 0.03 mg/l), Fe (0.146 ± 0.04 mg/l), Pb (0.071 ± 0.05 mg/l), and Zn (0.094 ± 0.10 mg/l). The average values of TDS, BOD, and COD in the effluent were found to be 6.3-, 9.3-, and 1.6-fold, respectively, higher than the permissible limit [25]. This result indicates the toxic nature of the CETP-treated effluent which may be a major source of environmental contamination. The effluent discharge from CETP will affect the aquatic ecosystem. The high values of TDS indicate the presence of minerals and toxic metals thus it may reduce the water clarity lesser the sunlight received by the water bodies lower the photosynthesis activity thus it harms the aquatic life [26,27]. Moreover, it will be effecting the osmoregulation function of the aquatic organism [28]. The dark brown colour of TE is mainly due to the presence of chromium, dye and other chemicals that are used during leather tanning process. [29]. The color obtained from chromium dyeing is an indicator of the presence of potentially inhibitory compounds and, in addition, may have a direct inhibitory effect on certain organisms lower in the food chain, chromium compounds have been found in the untreated and treated tannery. High levels of BOD and COD standards above the prescribed limits have harmful effects for aquatic organisms in the aquatic system and its low levels indicate suitable water quality which we can use in various sectors such as agriculture and industrial. The more oxygen is depleted in the stream which means less oxygen is available to higher forms of aquatic life then aquatic organisms become stressed and suffocate and die [15]. The high salt concentration of TEs due to the presence of sulphate, phosphate and nitrate, which were used during dehairing process of the skin, creates salinity in stream water and groundwater and it decreased survival and growth, increased osmolyte concentration in body fluids, and changed metabolic rates [3]. These substances are known to be hazardous, causing serious and long-term threats to humans, animals, and plants. When these chemicals interact with bacteria, inorganic, as well as other organic substances in waters, substituted compounds or other moieties can form, which are potentially as dangerous as the original phenolic compounds. Moreover, it may cause severe toxic, genotoxic, and carcinogenic effects on plants, animals, and humans [30]. Chromium is generally used in TIs for marking and surfacing of leather it is also used for dying leather products [31]. Higher chromium concentration causes fatal effects such as lymphocytosis, anemia, eosinophilia, bronchial, and renal lesions. Its high concentration can harm the gills of fish and also cause genotoxic and mutagenic effects on humans, plants, and animals [15,32].

Table 1: Physicochemical characteristics of tannery effluent collected from CETP Unnao

Parameters	Untreated	Treated	Permissible limits (CPCB, 2013)
Color	Dark brown	Light brown	-
pH	7.24±0.03	8.29±0.12	6.0-9.0
BOD	420±17.89	280±4.47	30.00
COD	1754±5.37	409±2.37	250.00
TS	25974±17.89	151812±37.86	Nil
TDS	23492±89.44	13317±2.68	2100.00
Total suspended solids	2482±17.89	1385.73±1.19	100.00
Phosphate	0.73±0.01	0.74±0.01	5.0
Sulfate	1062±17.89	3773.04±7.29	1000.00
Sulfide	2.2±0.18	2.3±0.24	2.00
Nitrate	0.097±0.002	734.86±0.42	10.00
Nitrite	0.8±0.09	735.55±1.28	Nil
Chloride	6116.21±4.82	8053.59±18.74	600.00
Fluoride	0.57±0.10	0.54±0.02	2.0
Oil and grease	1318.33±8.12	827.67±2.25	10.00
Thick solid	19150±9.47	14130.7±22.79	-
Volatile chemicals	6839.33±28.30	1052.67±10.78	-
Total nitrogen	1711.57±8.91	1788.27±7.81	-
Cr	81.6±0.18	7.2±0.02	2.00
Co	0.364±0.01	0.118±0.01	-
Ni	0.318±0.05	0.265±0.03	3.00
Fe	0.289±0.01	0.146±0.04	3.00
Pb	0.216±0.01	0.071±0.05	0.1
Zn	0.121±0.01	0.094±0.10	5.00

All values are in mg/l except color and pH. The results are reported as mean±standard deviation (SD) of triple samples

3.2. Bacteriological Analysis

In CETP-treated TE samples, the total heterotroph count and total coliform count were 2.29 x 1012 CFU/ml and 3.2 x 105 CFU/ml,

respectively. The results indicate that the concentrations of total heterotrophs and total coliforms were significantly higher in the treated TE sample. Therefore, the discharge of TE contaminated with a high bacterial load in the open environment may be responsible for waterborne diseases. Numberger [33] has been reported the presence of waterborne bacteria such as *Legionella, Leptospira, Mycobacterium,* and *Vibrio* in collected sample of wastewater treatment plant (WWTPs). Thus, existing effluent treatment techniques are inefficient for the proper eradication of bacterial load. Therefore, there is a reasonable need for improvements in treatment technology that can significantly reduce the bacterial load before discharge into the mainstream.

3.3. Antibiotic Susceptibility Assay

The results shown in Table 2 revealed that the indigenous heterotroph of TE was sensitive for amoxicillin, chloramphenicol, ciprofloxacin, gentamicin, norfloxacin, and tetracycline, and resistance to some antibiotics such as erythromycin, nalidixic acid, and penicillin-G. This investigation indicates that TE is an organically enriched medium and nutrients support the fast growth and spreading of multidrug- and multi-metal-resistant microbes in aquatic environments. Our results showed similarity with the findings of a previous study [24], which reported that *Enterococcus faecium* strain isolated from tannery sludge was resistant to erythromycin (15 µg/disc), vancomycin (30 µg/disc), kanamycin (30 µg/disc), and nalidixic acid (30 µg/disc). WWTPs are considered hotspots for antibiotic-resistant genes and for the spread of bacteria into the environment [33].

3.4. Recalcitrant Organic Pollutant Detected by GC–MS

GC–MS analysis of raw (untreated) and treated TE have been used to identify the organic pollutants. The organic pollutants identified from the NIST library in TE are shown in GC–MS chromatograms [Figure 1a and b and Table 3] and were primarily derivatives of fatty acids, organic acids, phenols, and endocrine-disrupting chemicals. However, in the treated TE, these compounds had disappeared [Figure 1b], and several new compounds were identified, which might be possible metabolites produced in CETP due to the bacterial degradation process during the secondary treatment process. Many organic compounds detected by GC-MS in raw effluents included were: 3-Hydroxyu-2-[(RS)-Ã -trimethylsilylbenzyl] butanoic acid (RT = 7.62), 1-(3Â'-Isopropylbicyclo[1.1.1] pent-1Â'-yl)-4- (TMS) benzene (RT = 15.83), docosanoic acid (RT=20.62), meso1,3,5-Trihydroxycyclohexane 1,5-diacetate (RT = 24.98), propanoic acid (RT = 32.99), decahydro-5, 8a-dimethyl-5-hydroxymethyl1-(3-hydroxypropyl)-2-naphthol (RT = 36.39), and dotriacontane (RT = 45.03). Further, in the chromatogram of treated effluent samples, the compounds with the RTs were methoxydimethyl(trimethylsilylmethyl) silane (RT = 7.60), methoxydimethyl(trimethylsilylmethyl)silane (RT = 12.65), hexadecane, (RT = 16.93), 1-Hexadecanol (RT = 20.57), sulfur (RT = 24.95), 1-Butyl-2-undecylcyclopropane (26.92), 1,2-Benzenedicarboxylic acid (RT = 30.91), 7-Methoxy-2,3-dihydro-2-phenyl-4-quinolone (RT = 32.96), and Ethyl 4,4,4-Trichloro-1-butenyl carbonate (RT = 36.35). The result of GC–MS analysis of raw and treated TE samples indicated that the CETP treatment process is not enough to degrade/remove the pollutants load because of its recalcitrant nature, and non-biodegradable pollutants with effluent were released into the environment. The United States Environmental Protection Agency has considered fatty acids, phenols, furans, alcohols, alkane hydrocarbons, aromatic hydrocarbons, and phthalates as "priority pollutants" [34,35].

3.5. Phytotoxicity Evaluation

The phytotoxicity of raw and treated TE was evaluated by observing seed germination and other plant growth parameter index on mung bean and fenugreek seeds. After 7 days of incubation, mung bean and fenugreek seeds exposed to raw and treated TE were monitored for various plant growth parameters such as germination %, root and shoot length, and biomass quantity, as mentioned in Tables 4 and 5.

Table 2: Antibiotic susceptible test results on isolated bacteria

Antibiotics used in study	Susceptibility pattern of the isolated bacteria
Ampicillin	I
Amoxicillin	S
Amikacin	I
Chloramphenicol	S
Ciprofloxacin	S
Erythromycin	R
Gentamicin	S
Neomycin	I
Nalidixic acid	R
Norfloxacin	S
Kanamycin	I
Polymyxin-B	I
Penicillin-G	R
Tetracycline	S
Vancomycin	I

S: Susceptible, R: Resistant, I: Intermediate

Table 3: Chemical compounds identified by GC–MS in raw (a) and CETP-treated (b) tannery effluents after extraction with dichloromethane

Retention time (RT)	Identified compound	a	b
7.60	Methoxydimethyl (trimethylsilylmethyl) silane	-	+
7.62	3-Hydroxyu-2-[(RS)-à-trimethylsilylbenzyl] butanoic acid	+	-
12.65	4-Benzyloxy-5-methoxybenzo[b] furan	-	+
15.83	1-(3'-Isopropylbicyclo[1.1.1]pent-1'-yl)-4-(TMS) benzene	+	-
16.93	Hexadecane	-	+
20.57	1-Hexadecanol	-	+
20.62	Docosanoic acid	+	-
24.95	Sulfur	-	+
24.98	meso-1,3,5-Trihydroxycyclohexane 1,5-diacetate	+	-
26.92	1-Butyl-2-undecylcyclopropane	-	+
30.91	1,2-Benzenedicarboxylic acid	-	+
32.99	Propanoic acid	+	-
32.96	7-Methoxy-2,3-dihydro-2-phenyl-4-quinolone	-	+
36.35	Ethyl 4,4,4-Trichloro-1-butenyl carbonate	-	+
36.39	decahydro-5,8a-dimethyl-5-hydroxymethyl-1-(3-hydroxypropyl)-2-naphthol	+	-
42.33	Unknown	-	+
45.03	Dotriacontane	+	+

The effect of different concentrations of raw and treated TE on seed germination of mung bean is shown in Figure 2. The observation of the seed germination test showed that the treated TE is less toxic as compared to the raw TE. Results from Table 4 revealed that the 100% seed germination was recorded at 6.25%–50% concentrations of raw and treated TE, whereas 100% effluent concentration of raw TE was showed no germination of mung bean seeds. The maximum root (5.40 ± 0.65 cm) and shoot length (8.20 ± 3.62 cm) were observed in the control as compared to all the concentrations of raw and treated TE. Among all concentrations of raw and treated TE, the 12.5% effluent concentration showed higher root and shoot length. However, the biomass quantity was not much changed if the seeds germinated in the raw. Surprisingly, the minimum biomass (0.09 ± 0.04 g) was recorded in treated effluent of 6.25% concentration. Moreover, the plant growth parameter index of mung bean is shown in Figure 3. Maximum GI % (70%) was recorded at a 12.5% concentration of raw TE. However, the higher concentrations of treated TE were less toxic as compared to raw TE concentrations for the GI% of mung bean seeds. Similar results patterns have been found in RRG%. The % phytotoxicity and relative toxicity % results revealed that there was not much significant change in the effect of raw and treated TE on mung bean seed germination.

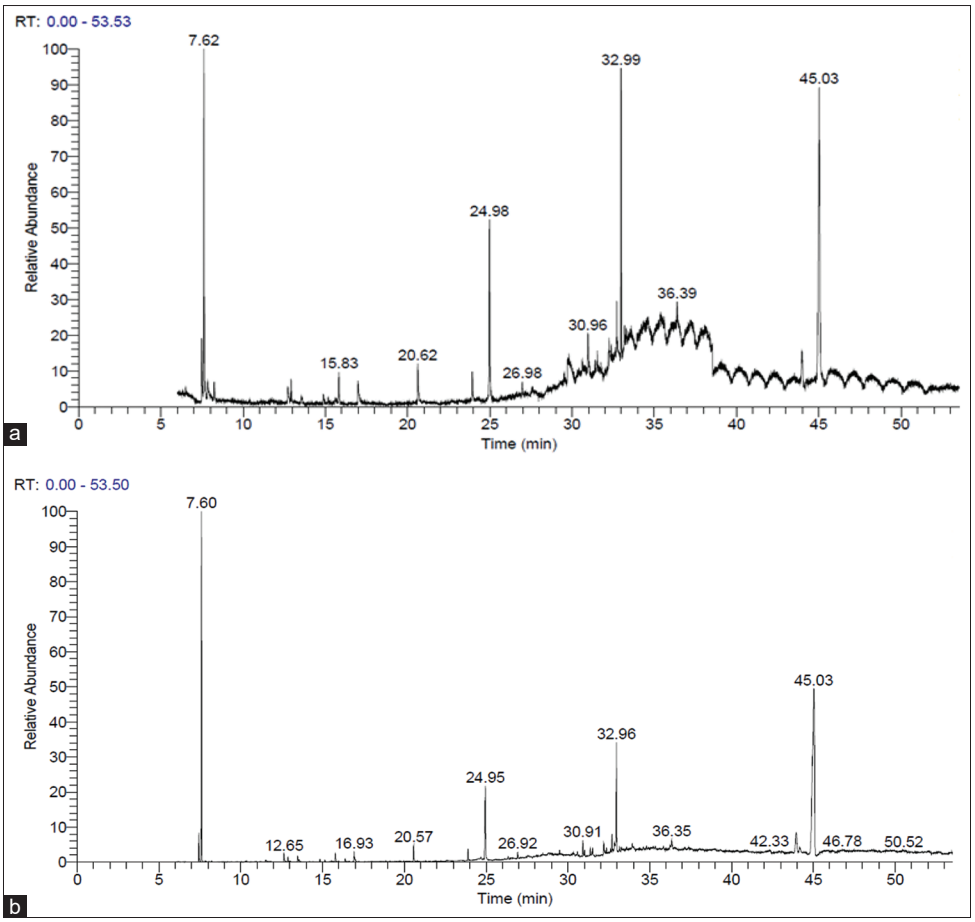

Figure 1: Typical chromatographic profile obtained by GC–MS of recalcitrant organic compounds extracted with DCM from untreated (a) and treated (b) tannery effluent sample

Table 4: Effect of different concentrations of raw and treated tannery effluents on seed germination, root and shoot length, and biomass of early seedling of mung bean (*Vigna radiata*) seeds

Treatments	Parameters							
	Germination %		Root length (cm)		Shoot length (cm)		Biomass (g)	
	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated
Control (DW)	100		5.40±0.65		8.20±3.62		0.22±0.06	
6.5%	100	100	1.60±1.02	2.62±1.52	1.76±0.75	2.62±0.82	0.18±0.02	0.09±0.04
12.5%	100	100	2.80±0.27	2.5±1.37	1.9±0.42	3.16±1.16	0.17±0.02	0.13±0.04
25%	100	100	1.62±1.26	1.98±0.34	1.26±0.51	2.7±0.57	0.18±0.02	0.15±0.02
50%	100	100	0.26±0.15	1.2±0.67	0.24±0.15	1.6±0.42	0.19±0.02	0.18±0.02
100%	00	90	0.00	0.26±0.15	0.00	0.4±0.19	0.00	0.16±0.02

Values are mean±SD of three samples

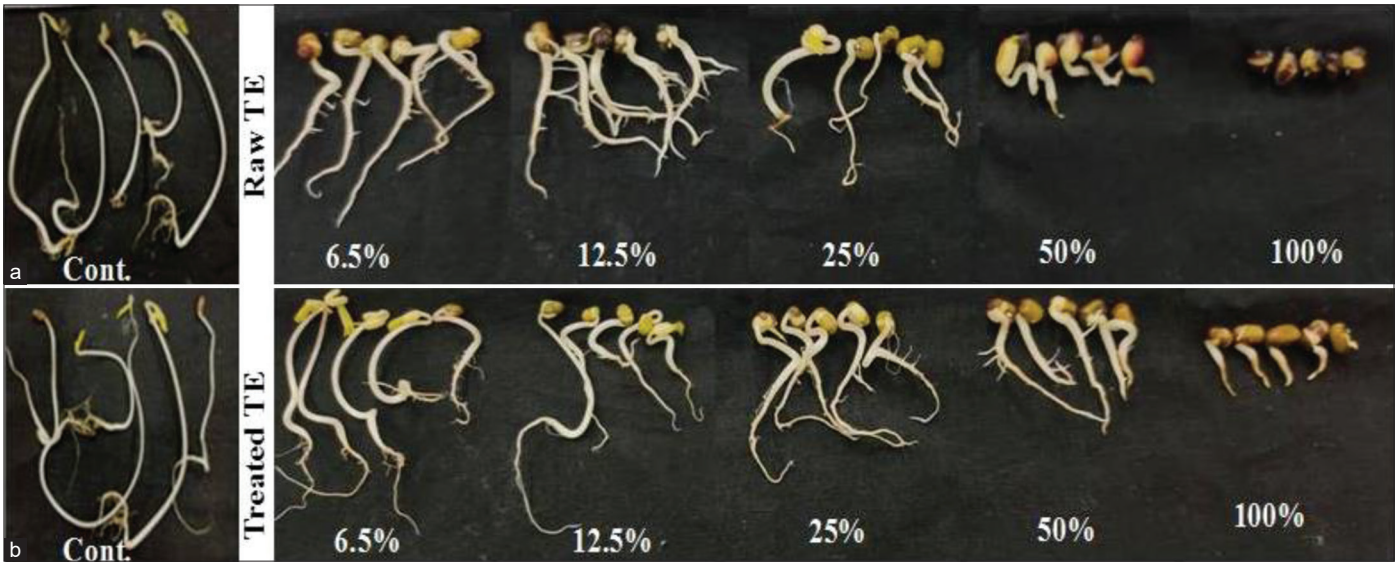

Figure 2: *Vigna radiata* seed germination in different concentration of (a) raw tannery effluent (TE) and (b) treated tannery effluent

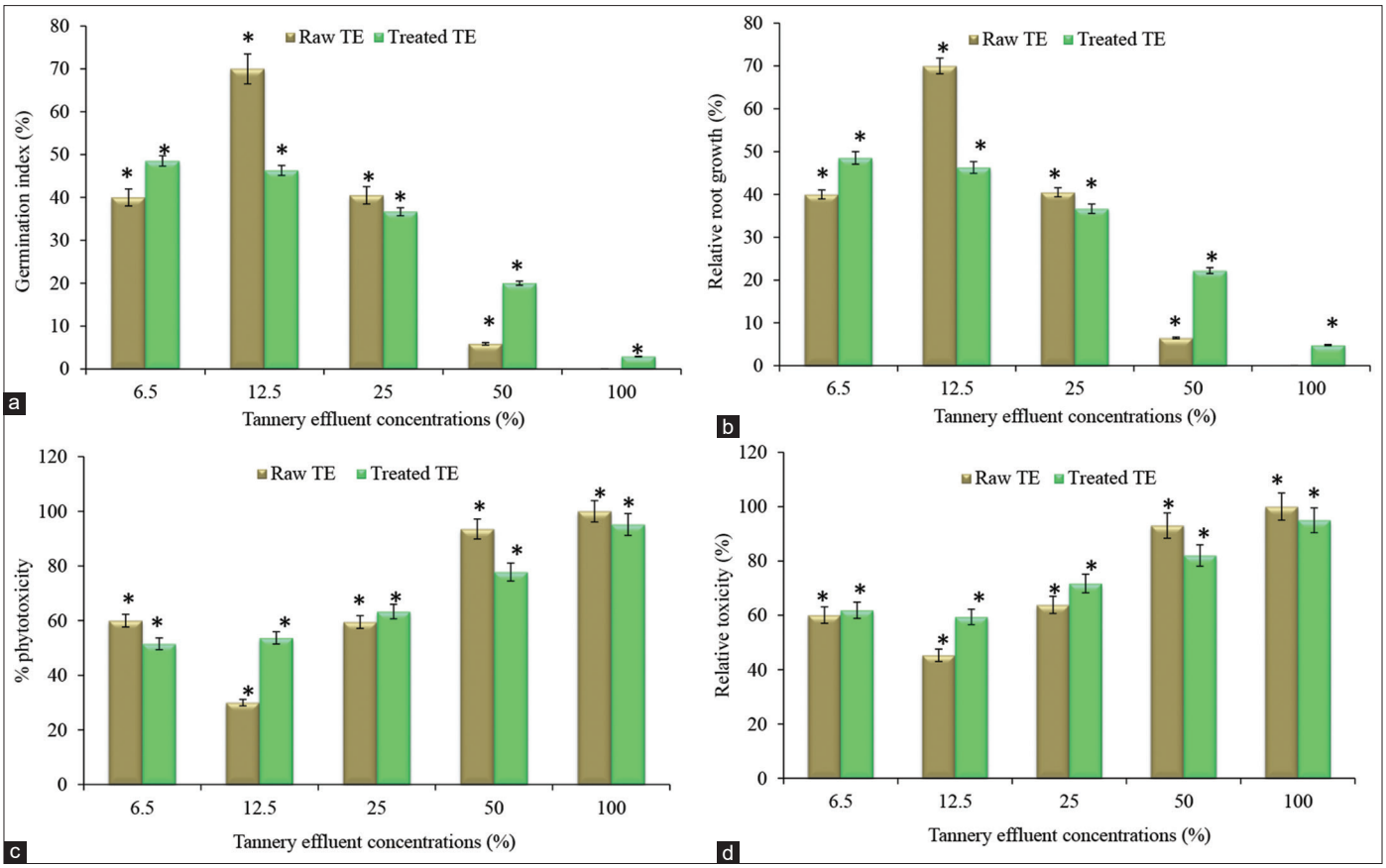

Figure 3: Phytotoxicity of different concentrations of raw and treated tannery effluents (TEs) on *Vigna radiata* seeds, (a) germination index (%), (b) relative root growth (%), (c) % phytotoxicity, and (d) relative toxicity (%), *denotes the p-value, $p<0.05$, significant when compared to control using ANOVA

The effect of different concentrations of raw and treated TE on seed germination of fenugreek is shown in Figure 4. The seed germination test results of fenugreek (*T. foenum-graecum*) seeds exposed with raw and treated TE are summarized in Table 5. The results showed that a 50% concentration of crude TE inhibited 100% seed germination in fenugreek seeds. However, 70% seedling growth was observed at 50% concentration of treated TE, whereas 100% seed inhibition was recorded when seeds were irrigated with 100% concentration of treated TE, resulting in 100% inhibition of seed germination. Maximum root (6.74 ± 0.94 cm) and shoot length (5.5 ± 1.0 cm) were recorded in control (DW). However, the root and shoot length of fenugreek were decreased gradually toward higher concentrations of

Table 5: Effect of different concentrations of raw and treated tannery effluents on seed germination, root and shoot length, and biomass of early seedling of fenugreek (*Trigonella foenum-graecum*) seeds

Treatments	Parameters							
	Germination%		Root length (cm)		Shoot length (cm)		Biomass (g)	
	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated
Control (DW)	100		6.74±0.94		5.5±1		0.38±0.01	
6.5%	100	100	1.34±0.74	0.96±0.42	1.84±0.39	2.48±0.61	0.05±0.01	0.05±0.01
12.5%	100	100	0.76±0.48	0.68±0.48	2.36±0.50	1.54±0.71	0.05±0.02	0.04±0.01
25%	50	70	0.16±0.15	0.56±0.35	0.28±0.28	1.08±0.11	0.04±0.01	0.05±0.02
50%	00	70	00±0.00	0.18±0.35	00±0.00	0.62±0.04	0.05±0.01	0.06±0.01
100%	00	00	00±0.00	00±00	00±0.00	0±0.00	00±0.00	00±0.00

Values are mean±SD of three samples

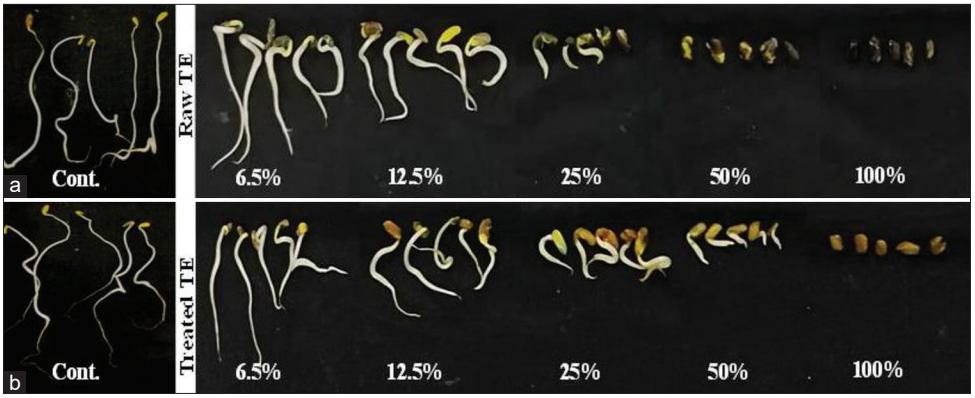

Figure 4: *Trigonella foenum-graecum* seeds germination in different concentration of (a) raw tannery effluent (TE) and (b) treated tannery effluent

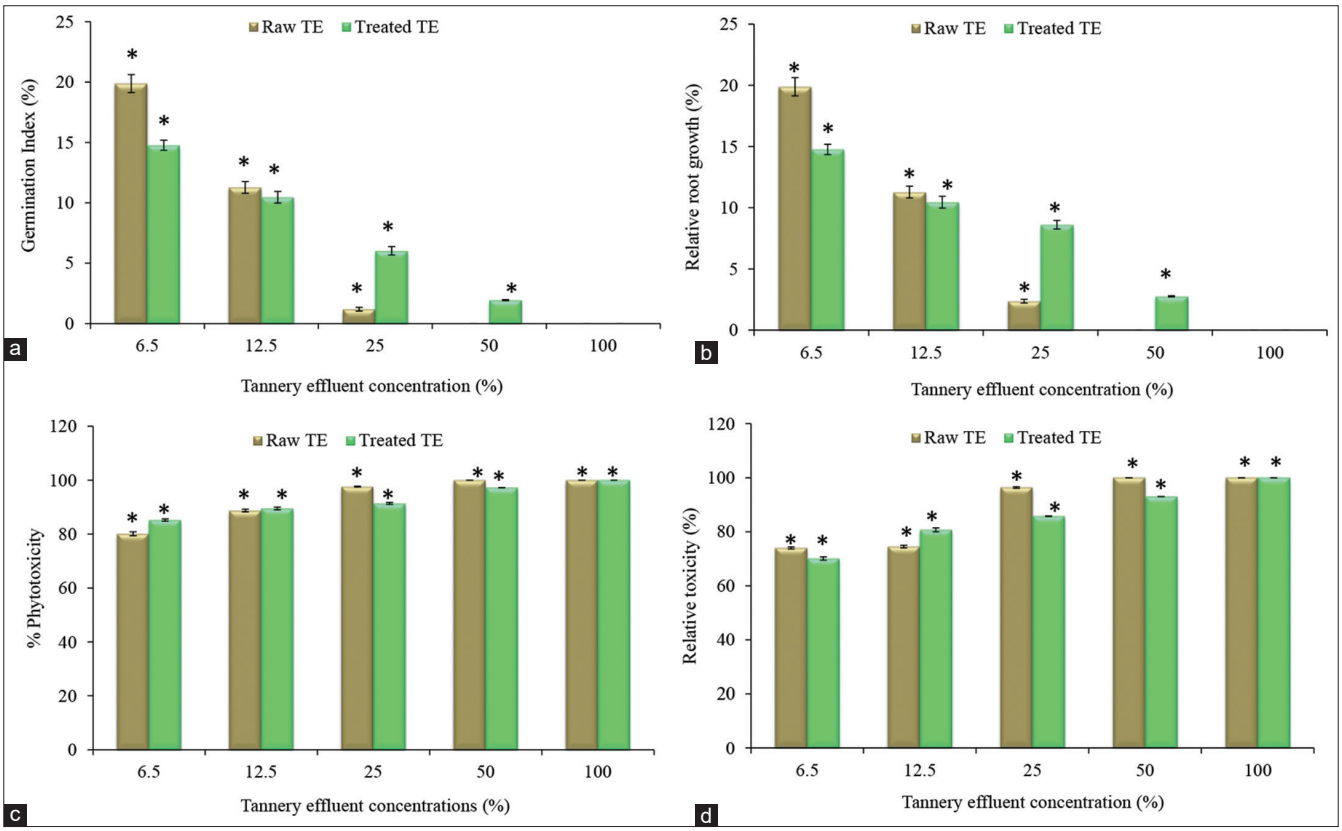

Figure 5: Phytotoxicity of different concentrations of raw and treated tannery effluents (TEs) on *Trigonella foenum-graecum* seeds, (a) germination index (%), (b) relative root growth (%), (c) % phytotoxicity, and (d) relative toxicity (%), *denotes the p-value, $p<0.05$, significant when compared to control using ANOVA

raw and treated TE. Noticeable biomass reduction was observed at all the concentrations of raw and treated TE when compared to control. Similarly, the GI% and RRG% were also reduced with increasing the concentrations of raw and treated TE [Figure 5]. Apart from this, the % phytotoxicity and relative toxicity % of raw and treated TE on fenugreek were increased with increasing the concentrations of effluent [Figure 5]. Overall, the phytotoxicity of raw and treated TE on mung bean and fenugreek seeds revealed that both effluents were toxic at the higher concentrations but their effect was greater on fenugreek seeds than mung bean seeds germination. The seed germination test is highly sensitive and proven to result in a very short time and it is also cost effective [36,37]. The previous studies also reported the toxicity of TE and domestic wastewater on fenugreek and mung bean seed germination [24,38]. The seed germination test was conducted for the evaluation of effluent quality. Hence, raw and treated TE was assessed for the phytotoxic effect on both seedlings. Due to the presence of high salts, phenolic compounds, or ROPs in the raw and treated TE, the germination of the seed is obstructed by the high concentration of TDS and Cr ion in wastewater causing osmotic stress and toxicity in the plant [39].

4. CONCLUSION

TE was collected from CETP, Unnao, for the analysis of pollution load and toxicity. The persistent organic pollutants were also analyzed by GC–MS. The analysis of TE revealed that effluents were loaded pollutants. The concentration of TDS, BOD, COD, and chromium was higher than the permissible limit fixed by regulatory bodies such CPCB, India. The GC–MS analysis showed that the toxic organic compounds were present in treated effluents. Further, the effluent was high as confirmed by phytotoxicity testing on fenugreek and mung bean plant models. These plant systems appear to be excellent indicators of the phytotoxicity and phytotoxic effects, leading to cellular and functional changes to cell death. The organic pollutants that remained in tannery wastewater after the secondary treatment process provide a chance for a variety of pathogenic and non-pathogenic microbes to flourish and contaminate the aquatic environments, whereas toxic metals induce genotoxic and mutagenic changes in bacterial communities making them resistant against a wide spectrum of antibiotics and toxic metals. In conclusion, the present study showed the presence of a plethora of contaminants in TE discharges by CETP which are toxic as determined by the phytotoxicity test. Hence, there is a need to implement effective remediation technology to safeguard public and environmental health due to contamination by TE.

5. ACKNOWLEDGMENT

Mr. Sandeep Kumar also acknowledges the encouragement and support given by Director, CSIR-IITR, for doing his PhD work. The author Annapurna Maurya acknowledges the University Grant Commission (UGC) for fellowship to carry out her Ph.D. work Special thanks to Dr Ratan Singh Ray (In charge of GC-MS facility) and Mr. GNV Satyanarayana, CSIR-IITR for GC-MS analysis. The author Abhay Raj acknowledges the financial support from the DST-SERB (Grant No.: EEQ/2017/000571). Dr. Dhirendra singh are also acknowledges the encouragement and support for his PhD work. This manuscript has been communicated under the CSIR-IITR communication number IITR/SEC/2021-2022/72.

6. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

7.AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

8.ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9.DATA AVAILABILITY

The study data is available with authors on request.

10.PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Kumar S, Yadav A, Maurya A, Pratap SG, Singh PK, Raj A. Characterization of tannery effluents by analyzing the recalcitrant organic pollutants and phytotoxicity assay. J App Biol Biotech. 2022;10(Suppl 2):91-99.
DOI: 10.7324/JABB.2022.10s210